



DIURETIC ACTIVITY OF IGNORED MONOCOT GRASS *KYLLINGA TRICEPS ROTTB*

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ABSTRACT

Kyllinga triceps rottb is commonly grown monocot grass of Gwalior Chambal region. In ayurvedic literatures it is known as *musta*. In various ethno botanical studies grass is reported to be diuretic. In present study we have been evaluated *kyllinga triceps* rhizomes for its diuretic, potential. Furosemide is taken as standard drug, both extracts petroleum ether and ethanolic exhibits significant diuretic action.

KEY WORDS

diuretic, ethanolic, extract, *kyllinga triceps*, petroleum ether.

INTRODUCTION

Herbal medicines are the use of plants and plant extracts as medicines. India is blessed with enormous varieties of medicinal and the aromatic plants, which may be attributed to the Indian climatic conditions. These plants are used as a potential source of many drugs in traditional Indian system of medicine.

The WHO is encouraging all the developing countries for using the traditional herbal medicines actively. There are 3000 plants used as medicine which are identified in the forests of India. The important chemical constituents from these plants are worth Rs 2000 crores in the US market. The plants are exhaustible source of medicine, but have remained unexplored. So, this provides a challenge for the medical and pharmaceutical sciences scientists for the search of new and potent drugs with minimal side effects. A lot of progress has been made in the phytochemical research from past few decades. Liver is an important organ of

our body and play a vital role in regulation of different physiological activities. It is involved in various vital functions like secretion, storage and metabolism. It detoxicates many toxic substances and synthesizes beneficial principles. The usage of plant-based preparation for curing the liver diseases, recommended in Ayurveda Medicinal plants have a rich treasure of drugs in treating liver disorders. Many plants act as hepatoprotective and some enhance immune system. Few drugs literally destroy the virus, induce the production of interferon, influence anti-inflammatory properties, lower the liver enzyme levels, decrease the fluid retention and increase the appetite. Mainly plant based preparations are used for treatment of liver distributed throughout India, Ceylon hot and warm temperate regions of the Old-World countries.

Plant is a major weed of improved pastures, but also occurs in crops, gardens, plantations and roadsides. It grows best in moist fertile soil that is seldom cultivated and in full sunshine. It is present in areas up to 7000 ft.

elevation. The plant is naturalized primarily in gardens and lawns.

The rhizomes of plant *Kyllinga triceps* rottb are fragrant, aromatic, sweet, astringent, bitter, refrigerant, febrifuge, antidiarrhoeal, diuretic, stomachic, anthelmintic, expectorant, demulcent and tonic. They are useful in vitiated conditions of pitta and vata, fever, cough, bronchitis, hepatopathy, splenopathy, diabetes, dermatitis, fistula and tumours.

MATERIALS AND METHODS

Plant Materials

The species for the proposed study that is *Kyllinga triceps* rottb were collected from Bhoora Khon area of Shivpuri District of Gwalior Division (M.P.) with the help of Mr. N.K. Pandey (R.O.) National Research institute for ayurvedic-siddha (CCRAS) Amkho, Gwalior.

The species for the proposed study was identified as *Kyllinga triceps* by Dr. (Smt.) M.D. Gupta (Asst Director) and Mr. N.K. Pandey (R.O.) National Research Institute for Ayurveda and siddha (C.C.R.A.S.) under Ministry for Health and Family Welfare, Govt. of India, Amkho Gwalior (M.P.)

Preparation of Plant Extract

Preparation of the extract of *Kyllinga triceps* rottb, powdered rhizome is done by using following solvents. Ethanolic extract cold percolation method was used for preparation of 80% alcoholic extract of dried *Kyllinga triceps* rottb, rhizomes powder, rhizome powder were extracted with 80% ethanol for 24 hrs, which was filtered with 80 mesh nylon cloth. Rawmaterial and solvent ratio was 1:8, total extraction procedure was repeated for five times, clean and sterile conditions were maintained throughout the extraction process so that there should be no chance of contamination. All the filterates obtained after extraction were combined and again subjected for filtration with 250 mesh nylon cloth, finally extract was obtained was concentrated with reduced pressure.

Petroleum Ether extract cold percolation method was used for preparation of petroleum ether extract of dried *Kyllinga triceps* rottb, rhizomes powder, rhizome powder were extracted with Petroleum ether for 24 hrs, which was filtered with 80 mesh nylon cloth. Rawmaterial and solvent ratio was 1:8, total extraction procedure was repeated for five times, clean and sterile conditions were maintained throughout the extraction process so that there should be no chance of

contamination. All the filterates obtained after extraction were combined and again subjected for filtration with 250 mesh nylon cloth, finally extract was obtained was concentrated with reduced pressure.

Experimental animals

Albino rats weighing between 175-200 g of either sex were used in the study, The experimental protocol was approved by the Institutional Animal Ethical Committee, and these animals were used to evaluate the diuretic activity of Ethanolic and petroleum Ether Extracts of the plant *Kyllinga triceps* rottb. The animals were maintained under standard husbandry conditions for an acclimatization period of 15 days before performing the experiments. All rats were housed in metallic cages six in each and temperature maintained at $22\pm 2^{\circ}\text{C}$.

Drug used

Furosemide 10 mg/ml (Sanofi Aventis, Andheri East, Mumbai).

Experimental model

Lipschitz test

The method of lipschitz et.al was employed for the assessment of diuretic activity. Six groups of 6 albino Rats, each weighing 175-200 g were fasted and deprived of water for 18 hours prior to the experiments. On the day of the experiment all the animals were given normal saline orally 25 ml/kg body weight.

Group –I-Control

Group –II- Furosemide (10 mg/kg) standard

Group –III- Petroleum Ether Extract (100 mg/kg)

Group – IV- Petroleum Ether Extract (200 mg/kg)

Group – V- Ethanolic Extract (100 mg/kg)

Group – VI- Ethanolic Extract (200 mg/kg)

Immediately after dosing, the animals were placed in metabolic cages specially designed to separate urine and faeces and kept at room temperature of $25 \pm 0.5^{\circ}\text{C}$. The urine was collected in measuring cylinder, every 30 minutes for 5 hours in all groups and at the end of 5th hour the collected urine stored at -20°C until further analysis.

During this period no water or food was made available to the animals. The total volume of urine collected was measured for the control and treated groups. The parameters taken for each individual rat were body weight total urine volume urine concentrations of Na^+ , K^+ , Cl^- , Na^+ and K^+ concentration were measured by flame photometry and Cl^- concentrations was estimated titrimetrically. To obtain diuretic activity, the

diuretic action of the extract was compared to the diuretic action of the standard drug in the test group (Formula – 3) (Mukherjee., 2000).

$$\text{Urinary Excretion} = \frac{\text{Total Urinary Output}}{\text{Total Liquid Administered}} \times 100\%$$

$$\text{Diuretic Action} = \frac{\text{Urinary Excretion of Treatment Groups}}{\text{Urinary Excretion of control}}$$

$$\text{Diuretic Activity} = \frac{\text{Diuretic Action of Treatment group}}{\text{Diuretic Action of Standard group}}$$

Estimation of urinary electrolytes

Sodium, potassium and chloride levels of urine and the plant extract were analyzed. Sodium and potassium concentrations were determined by making use of flame photometry, and chloride concentration was quantified using Ion Selective Electrode (ISE) analyzer (AVL 9181 Electrolyte Analyzer, Roche, USA). The flame photometer worked by flame production when the atom changed from its excited state to the ground state, while the ISE analyzer contains software which permits electrolyte parameter configuration. A calibration was performed automatically in both cases prior to analysis with different levels of standards. Ratios of electrolytes; Na^+/K^+ and $\text{Cl}^-/\text{K}^++\text{Na}^+$ were calculated to evaluate the Sal uretic activity of the different extracts. In addition, pH was directly determined on fresh urine samples using a pH meter. Moreover, the salt content of the extract was also determined to rule out its contribution on urinary electrolyte concentration.

Statistical analysis

The experimental results were expressed as the mean \pm standard error of mean and the statistical significance was evaluated by using students' t' test.

RESULT AND DISCUSSION

Diuretic activity:

Effect on Urine Volume

❖ Petroleum Ether extract

The petroleum ether extract of the plant produced diuresis, which appeared to be a function of dose (Table 1). The time course of action of diuresis is also depicted in Figure 2. PE-KTR 100 mg/kg did not produce any detectable difference in urine volume compared to CON animals at all time points. PE-KTR 200 mg/kg started to increase urine volume from the second hour, but changes reached statistical significance at the fourth (68%, $p < 0.05$)

and fifth (93.3%, $p < 0.01$) hour. F10 treated mice exhibited a significantly greater urine volume from the first hour (90%, $p < 0.01$), which continued until the end of the fifth hour (95%, $p < 0.01$). F10 displayed a significantly greater effect compared to PE-KTR 100 mg/kg ($p < 0.001$) throughout the time points, but had no significant difference with that of PE-KTR 200 mg/kg from the second hour onwards.

❖ Alcoholic extract

No apparent difference was observed between controls and mice treated with the first two doses (AE - KTR 100 mg/kg and AE - KTR 200 mg/kg) at all time points. F10, on the other hand, produced a significant increase starting from the first hour. Both AE - KTR 100 mg/kg ($p < 0.001$) and AE - KTR 200 mg/kg ($p < 0.01$) produced a lower diuretic effect as compared to that of Fig.

Electrolyte content of the extract

Water soluble salts could be present in the extract and subsequently interfere with the urinary excretion of electrolytes. The content of Na^+ , K^+ and Cl^- in both extracts was therefore determined to exclude this possibility. The result revealed that Na^+ and Cl^- were not detectable at all doses in the extracts as tested by the instrument used in the present study. K^+ content of the petroleum ether extract was found to be 35.3 and 40.7 mmol/l for PE-KTR 100 mg/kg and PE-KTR 200 mg/kg respectively. In case of alcoholic extract, values 12 and 20 mmol/l was detected for AE - KTR 100 mg/kg, and AE - KTR 200 mg/kg respectively.

Urinary pH

Urinary pH measurement revealed that the different treatment groups of both petroleum ether and alcoholic extracts had produced relatively alkaline urine. The pH of urine treated with the petroleum ether extract had shown an increase from PE-KTR 100 mg/kg (7.4) to PE-KTR 200 mg/kg (7.8). The CON group produced the lowest pH and the standard group an intermediate pH (7.8) between vehicle and extract treated groups. Treatment with the alcoholic extract increased pH from 7.3 (AE - KTR 100 mg/kg) to 7.6 (AE - KTR 200 mg/kg). Urinary pH of F10 was higher than the lowest doses but Similar to the high dose (Figure 47).

Table 01: Effect of Petroleum Ether and alcoholic extracts on 5 h urine volume

Group	Volume of urine (ml)					Diuretic action	Diuretic activity
	1 h	2 h	3 h	4 h	5 h		
CON	0.62 ± 0.13	0.81 ± 0.11	0.97 ± 0.11	1.0 ± 0.11	1.05 ± 0.10	1	
F10	1.18 ± 0.07 ^{a1}	1.46 ± 0.09 ^{a3}	1.64 ± 0.08 ^{a1}	1.79 ± 0.09 ^{a2}	2.05 ± 0.11 ^{a2}	1.95	1
PE-KTR 100 mg/kg	0.38 ± 0.04 ^{b3,c3}	0.69 ± 0.05 ^{b3,c2}	0.98 ± 0.17 ^{b2}	1.11 ± 0.18 ^{b2,c1}	1.16 ± 0.17 ^{b3,c2,d1}	1.1	0.56
PE-KTR 200 mg/kg	0.56 ± 0.08 ^{b3,c2}	1.09 ± 0.07	1.41 ± 0.12	1.68 ± 0.13 ^{a1}	2.03 ± 0.13 ^{a2}	1.93	0.99
AE-KTR 100 mg/kg	0.45 ± 0.46 ^{b3,c2}	0.62 ± 0.07 ^{b3}	0.68 ± 0.72 ^{b3,c2}	0.84 ± 0.07 ^{b3,c2}	1.1 ± 0.85 ^{b3,c3}	1.06	0.54
AE-KTR 200 mg/kg	0.48 ± 0.09 ^{b3,c1}	0.71 ± 0.09 ^{b3}	0.91 ± 0.12 ^{b3}	1.08 ± 0.13 ^{b2,c1}	1.31 ± 0.11 ^{b2,c2}	1.25	0.64

(n = 6); For petroleum ether extract: ^aagainst control, ^bagainst standard, ^dagainst PE-KTR 200 mg/kg mg/kg, ^eagainst PE-KTR 100 mg/kg, ^fagainst AE - KTR 100 mg/kg, ^gagainst AE - KTR 200 mg/kg; For alcoholic extract ^aagainst control, ^bagainst standard; ¹p < 0.05, ²p < 0.01, ³p < 0.001; PE refers to petroleum ether extract and AE to alcoholic extract; F10: Furosemide 10 mg/kg; CON: controls treated with distilled water; Numbers refer dose in mg/kg.

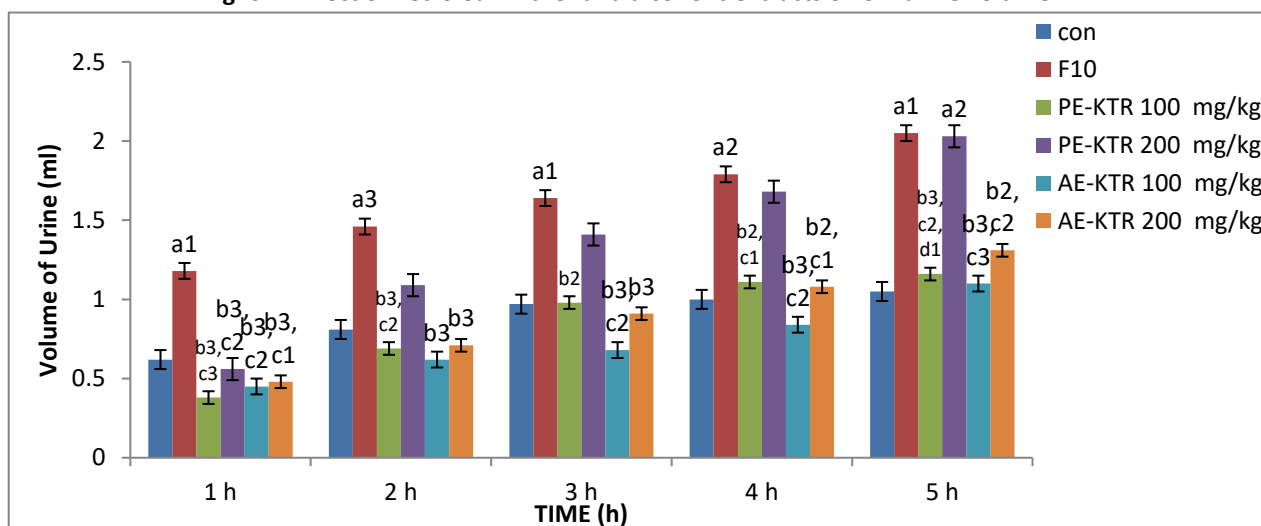
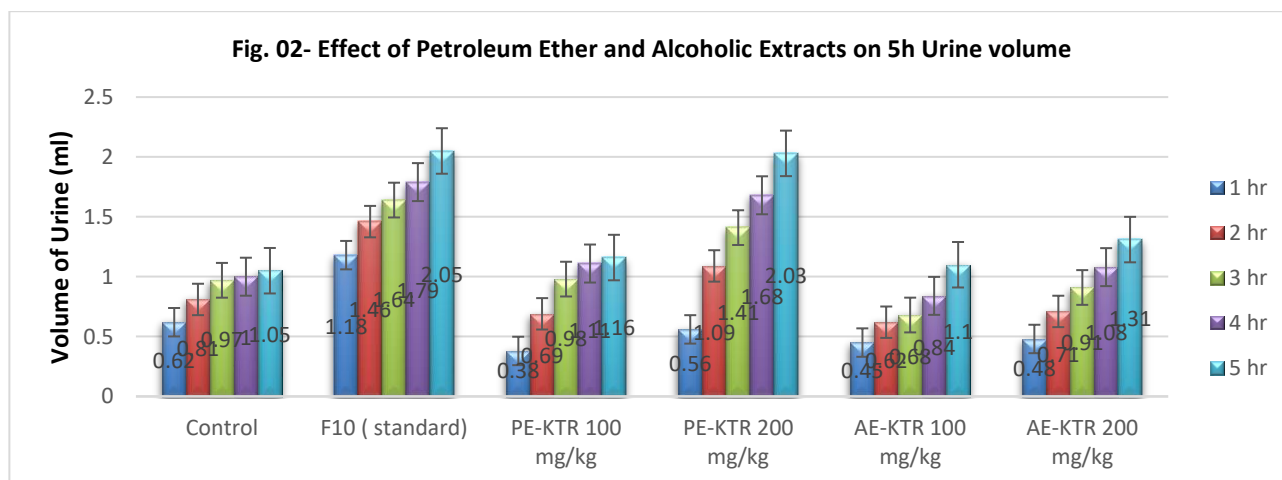
Fig. 01: Effect of Petroleum Ether and alcoholic extracts on 5 h urine volume

Fig. 02- Effect of Petroleum Ether and Alcoholic Extracts on 5h Urine volume


Figure 02: Time course of diuresis in rats treated with different doses of petroleum ether and alcoholic extract: n = 6, PE-KTR 100 mg/kg, PE-KTR 200 mg/kg, AE-KTR 100 mg/kg and AE-KTR 200 mg/kg; F10- Furosemide 10 mg/kg, CON – group that received distilled (5 ml/kg) water.

CONCLUSION

Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria, and cirrhosis of liver. Since diuretics are employed clinically in the treatment of edema, it would be highly important to demonstrate effectiveness in the presence of electrolyte and water. Thus, it is presumed to be advantageous to 'pre-treat' or 'prime' the test animals with various fluids in screening agents for potential diuretic activity. The petroleum ether and alcoholic extracts showed an increase in urine volume that appeared to vary with dose and time as well as the nature of the extract. Compared to the alcoholic extract, the petroleum ether extract produced a better diuretic effect. The lower doses of both extracts did not produce an appreciable effect, but, whilst the high dose of the petroleum ether extract was able to produce significant effect beginning from the fourth hour, the same dose of the alcoholic extract was devoid of any effect until the end of the observation period. This could probably suggest that the lower doses might represent subthreshold doses. The higher dose of both the PE and alcoholic extracts produced comparable effect to that of furosemide. Even better effect was observed with the petroleum ether extract, as it had a diuretic activity even more than the standard drug. Diuretic activity is considered to be good if it is more than 1.50, moderate

if it is 1.00-1.50, little if it is between 0.72-1.00 and nil if it less than 0.72.

The effect of the extracts on water excretion was accompanied by urinary electrolyte excretion effect, since there appeared to be an increased salt excretion as compared to the control group, which supports the idea that the diuretic effect was of the saluretic type in contrast to aquaretic type, which is a typical feature of most phytodiuretic agents. The larger doses of both extracts did have an interesting natriuretic effect and thus could have a beneficial effect in different edematous conditions. The results indicate that the extracts increase sodium excretion more than potassium, which is considered as a very good safety profile of diuretic agents, as hypokalemia is one of the potential adverse effects of synthetic diuretics, such as furosemide.

In determination of urinary pH, the extracts showed a relative increase in pH values as compared to controls, reinforcing the notion that carbonic anhydrase inhibition as one of the possible mechanisms of action of the plant. In addition, the reduction of potassium excretion at the maximum doses of the extracts along with the resulted alkalinizing of urine might give a clue on the possibility of the plant acting as a modest potassium-saving diuretic. Diuretic activity is considered to be good if it is more than 1.50, moderate if it is 1.00-1.50, little if it is between 0.72-1.00 and nil if it less than 0.72.

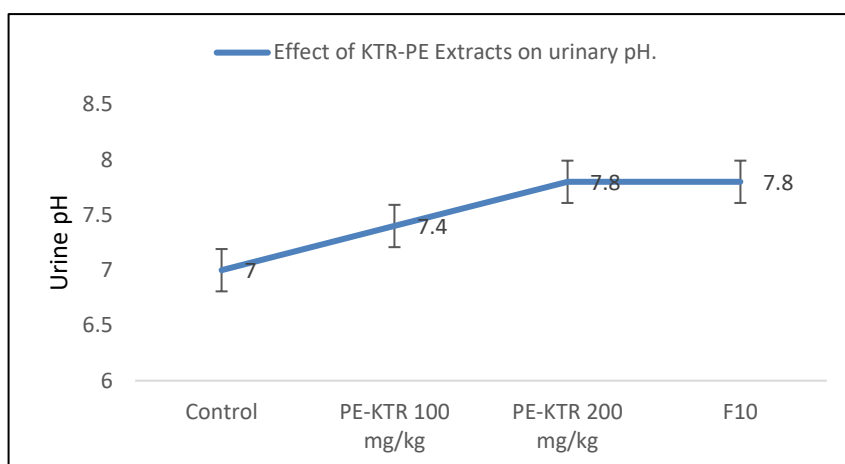
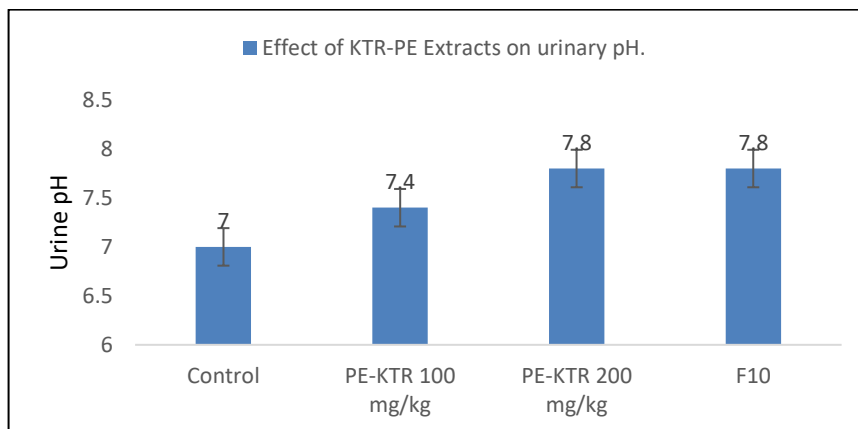
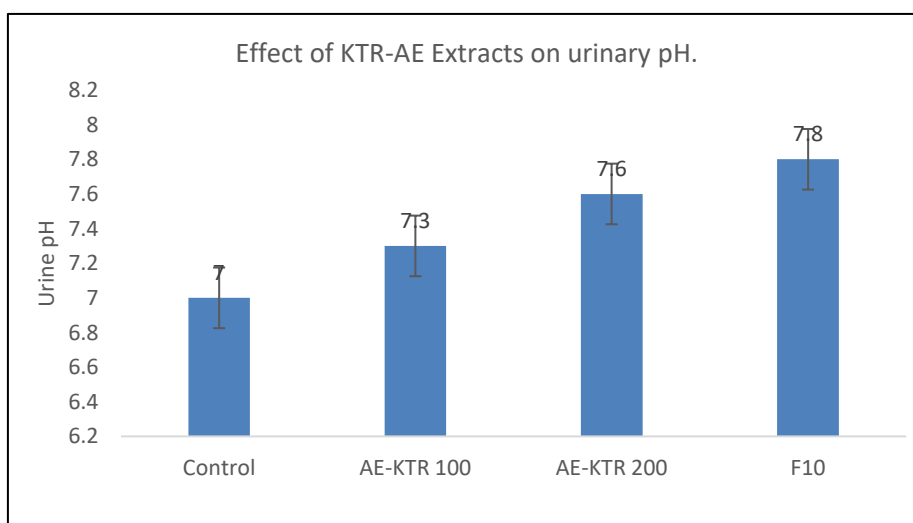
Table 2: Effect of Petroleum ether and alcoholic extracts on 5 h urinary electrolyte excretion in rats.

GROUP	Urinary electrolyte concentration(mmol/L)			Saluretic index			Na ⁺ /K ⁺	Cl ⁻ /Na ⁺ +K ⁺
	Na ⁺	K ⁺	Cl ⁻	Na ⁺	K ⁺	Cl ⁻		
CON	59.97 ± 7.83	43.7 ± 5.80	44.2 ± 2.58				1.37	0.43
F10	101.3 ± 6.01 ^{a3}	92.07 ± 13.85 ^{a3}	73.85 ± 11.27	1.68	2.1	1.67	1.1	0.38
PEKTR100	90.37 ± 5.8 ^{a1,e1}	97.5 ± 13.2	55.74 ± 6.23	1.50	2.23	1.26	0.92	0.29
PEKTR200	101.7 ± 4.72 ^{a2,e2}	100.6 ± 11.7	56.82 ± 8.06	1.69	2.3	1.29	1.01	0.28
AEKTR100	49.70 ± 2.74 ^{b3}	57.5 ± 5.94 ^{b1}	50.87 ± 10.58	0.83	1.32	1.13	0.86	0.47
AEKTR200	80.62 ± 4.89	72.85 ± 2.66	58.75 ± 8.34	1.34	1.67	1.33	1.11	0.38

(n = 6); For petroleum ether extract: ^aagainst control, ^bagainst standard, ^dagainst PE-KTR 200 mg/kg mg/kg, ^eagainst PE-KTR 100 mg/kg, ^fagainst AE - KTR 100 mg/kg, ^gagainst AE - KTR 200 mg/kg; For alcoholic extract ^aagainst control, ^bagainst standard; ¹p < 0.05, ²p < 0.01, ³p < 0.001; PE refers to petroleum ether extract and AE to alcoholic extract; F10: Furosemide 10 mg/kg CON: controls treated with distilled water; Numbers refer dose in mg/kg.

Table.3. Effect of *Kyllinga triceps rottb.* Extracts on urinary pH.

Control	F10	PE-KTR 100	PE- KTR 200	AE-KTR 100	AE-KTR 200
pH-7	7.8	7.4	7.8	7.3	7.6


Figure 03: Urinary pH of rats treated with Petroleum ether extract: PE-KTR 100 mg/kg, and PE-KTR 200 mg/kg; F10- Furosemide 10 mg/kg, CON –group that received distilled (5 ml/kg) water.


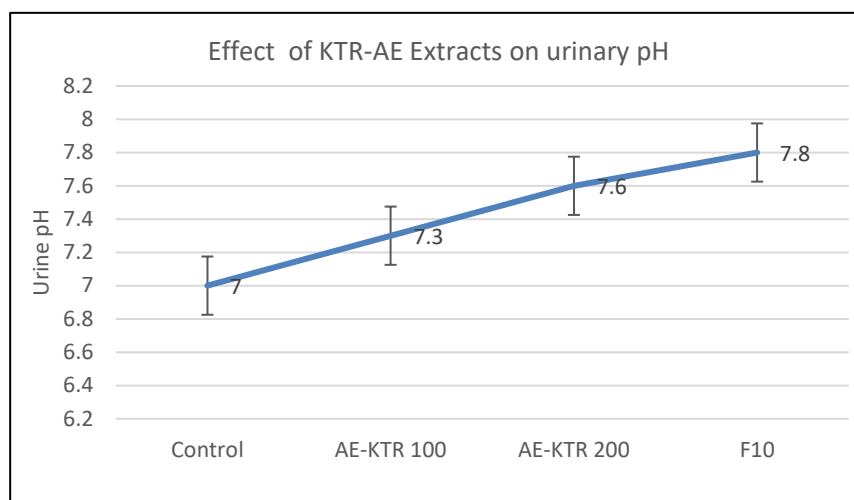


Figure 4: Urinary pH of rats treated with Alcoholic extract: AE-KTR 100 mg/kg, and AE-KTR 200 mg/kg; F10-Furosemide 10 mg/kg, CON –group that received distilled water.

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